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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.

If no title is shown please refer to the description.

Si aucun titre n'est indiqué se référer à la description.)

Diagnostic and therapeutic use of steroidogenic acute regulatory protein for  
neurodegenerative diseases

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## **DIAGNOSTIC AND THERAPEUTIC USE OF STEROIDOGENIC ACUTE REGULATORY PROTEIN FOR NEURODEGENERATIVE DISEASES**

The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social and economic burden. AD is the most common age-related neurodegenerative condition affecting about 10 % of the population over 65 years of age and up to 45 % over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with AD are senile plaques, composed of amyloid- $\beta$  protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles. AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-192).

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon4 allele of apolipoprotein E (ApoE). Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP), presenilin-1, and presenilin-2, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurological diseases and to provide methods, materials, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

The brain is a target site of peripheral steroid hormones synthesized and secreted into circulation by the adrenal glands, gonads and the fetoplacental axis. It is well-known, for instance, that gonadal androgens act on the brain to influence the reproductive behaviour of individuals. However, the brain is also a site of synthesis of steroid hormones, a process termed neurosteroidogenesis. Only two decades have passed from the first description of dehydroepiandrosterone sulfate production in rodent brain (Corpechot et al., *Proc. Natl. Acad. Sci. U.S.A.* 1981, 78:4704-4707) and, therefore, this field of research is as a young discipline at the interface of neurology, endocrinology, and behavioral sciences.

There are two principle categories of steroid hormone action in brain tissue, a genomic action and an immediate non-genomic action. At the genomic level, steroid hormones bind to intracellular/nuclear hormone receptors that regulate transcription of target genes. These adaptive responses generally occur

within hours or days. In addition, steroid hormones show an immediate impact on the excitability of the central nervous system through action on neurotransmitter-gated ion channels (for review: Stoffel-Wagner, *Eur. J. Endocrinol.* 2001, 145:669-679). In particular, pregnenolone sulfate potentiates the calcium conductivity of N-methyl-D-aspartate receptors and suppresses the chloride conductivity of the gamma-aminobutyric acid receptors in rat hippocampal neurons, thus affecting the overall neuronal excitability (Irwin et al., *Neurosci. Lett.* 1992, 141:30-34; Harrison et al., *J. Neurosci.* 1987, 7: 604-609).

In order to understand the physiological roles of neurosteroidogenesis in the mammalian brain it is important to identify the brain regions involved and the type(s) of cells that are proficient for the key enzymes and therefore likely to be active in the synthetic process. Most of the current knowledge in this regard is derived from studies in rodents, and the overall picture that emerges is that glial cells, oligodendrocytes and astrocytes play a major role in neurosteroid formation and metabolism in cerebrum and cerebellum (for review: Tsutsui et al., *Neurosci. Res.* 2000, 36:261-273). In more recent studies in the rat system, on the other hand, pyramidal and granule neurons of the hippocampus (Furukawa et al., *J. Neurochem.* 1998, 71:2231-2238; Kimoto et al., *Endocrinology* 2001, 142:3578-3589) and Purkinje cell neurons of the cerebellum (Ukena et al., *Endocrinology* 1998, 139:137-147; Ukena et al., *Endocrinology* 1999, 140:805-813) were shown to possess the key enzymes of neurosteroidogenesis as well. Due to the difficulty of obtaining fresh brain material, analogous studies in the human system are in their infancy. Using a sensitive reverse transcriptase polymerase chain reaction approach, the messenger RNA for the rate-limiting enzyme of neurosteroidogenesis, CYP11A1, was detected in surgical specimen of human brain cortices of the frontal and temporal lobes and the hippocampus, albeit at levels 200 times lower than in the adrenal gland (Watzka et al., *J. Neuroendocrinol.* 1999, 11: 901-905). Messenger RNAs for two other key enzymes of neurosteroidogenesis, CYP21 and 17-beta hydroxysteroid dehydrogenase, were also detected in surgical specimen of the temporal lobe

and hippocampus (Beyenburg et al., *Neurosci. Lett.* 2001, 308:111-114; Stoffel-Wagner et al., *J. Endocrinol.* 1999, 160:119-126). Based on this small complement of enzymes, seven different steroids can principally be synthesized in situ, including pregnenolone and progesterone. However, additional neurosteroidogenic enzymes have been detected in brain, including CYP19 (aromatase), 5-alpha-reductase, 3-alpha-hydroxysteroid dehydrogenase, CYP17, CYP11B1 and others (for review: Stoffel-Wagner, *Eur. J. Endocrinol.* 2001, 145:669-679) and, therefore, many more neurosteroids are likely synthesized within the brain.

To a large extent the potential physiological and pathophysiological implications of the above findings remain to be elucidated. For instance, the anticonvulsive, anesthetic, and anxiolytic effects of neuroactive steroids are attributed to their capacity to modulate the gamma-aminobutyric acid receptor function and are therefore of immediate, non-genomic nature (Stoffel-Wagner, *Eur. J. Endocrinol.* 2001, 145:669-679). Furthermore, it has been postulated that neurosteroids act on nerve cells through membrane receptors coupled to G proteins; various neuropeptide receptors and progesterone receptors (Orchinik et al., *Proc. Natl. Acad. Sci. U.S.A.* 1992, 89:3830-3834; Grazzini et al., *Nature* 1998, 392:509-512; Rupprecht et al., *Neuron* 1993, 11:523-530). On a more phenomenological level, the abundant neurosteroids dehydroepiandrosterone and dehydroepiandrosterone sulfate, generated from pregnenolone by action of cytochrome P450 17-alpha-lyase and hydroxysteroid sulfotransferase, are discussed as neuroprotective agents, whose levels decrease with age and under stress (Kimonides et al., *Proc. Natl. Acad. Sci. U.S.A.* 1998, 95:1852-1857). A potential role of pregnenolone, dehydroepiandrosterone and their sulfate esters in learning, memory and cognitive aging is under controversial discussion (Vallee et al., *Brain Research Reviews* 2001, 37:301-312; WO 01/55692). Fluctuations in neuroactive steroids are thought to contribute to the increased risk of developing psychiatric diseases in women during the perimenstrual phase, pregnancy, the post partum period, and menopause. On the cognitive level, estrogens and androgens impact on verbal fluency, the performance of spatial

tasks, verbal memory tests, and fine-motor skills (for review: McEwen, *Annu. Rev. Neurosci.* 1999, 22:105-122; Stoffel-Wagner, *Eur. J. Endocrinol.* 2001, 145:669-679).

The instant invention discloses the differential expression of the steroidogenic acute regulatory protein (StAR) gene in different regions of the AD brain but not in the brain of healthy age-matched control individuals. StAR is encoded by the synonymous gene on human chromosome 8p11.2 (Sugawara et al., *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92:4778-4782; GenBank accession number U17280; US patent 6194555; US patent 5807678). StAR is a 285 amino acids protein partly located in mitochondria and synthesized as a conformationally labile precursor with an N-terminal mitochondrial targeting sequence on cytoplasmic ribosomes (Stocco, *Biochim. Biophys. Acta* 2000, 1486:184-197). By virtue of its so called START domain, StAR stoichiometrically binds cholesterol within the cytoplasm and shuttles the hydrophobic cargo through both mitochondrial membranes and the enclosed intermembrane space. Within the inner mitochondrial membrane cholesterol is transferred to CYP11A1 which then catalyzes the conversion of cholesterol to pregnenolone as the committed step of neurosteroidogenesis (Stocco, *Biochim. Biophys. Acta* 2000, 1486: 184-197). Multiple transcripts of StAR were found in human steroidogenic tissues, in particular ovary, testis, adrenal gland, and within the kidney (Sugawara et al., *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92:4778-4782). However the same study did not identify StAR expression in human brain (ibid.). Expression of StAR is under control of the acute regulation by tropic hormones that generally act via the cAMP second messenger system. In case of the adrenal and gonads these tropic hormones are pituitary gland derived corticotropin and luteinizing hormone, while in the kidney, expression of StAR responds to parathyroid hormone (Sugawara et al., *Proc. Natl. Acad. Sci. U.S.A.* 1995, 92:4778-4782). In addition to regulation of gene expression, StAR protein activity or stability is thought to be regulated by cAMP dependent protein kinase / protein kinase A (Clark et al., *Endocrine Res.* 2000, 26:681-689).

Mutations in the StAR gene have been associated with severe human disease conditions termed lipid congenital adrenal hyperplasia and dose sensitive sex reversal (Lin et al., *Science* 1995, 267:1828-1831; Tee et al., *Hum. Molec. Genet.* 1995, 4:2299-2305; US patent 6194555, US patent 5872230, US patent 5807678 / WO 9629338). More recently, overexpression and/or amplification of the StAR gene was discovered in a number of types of cancer cells, in particular breast cancer cells (WO 131342). To date, no mutations in the StAR gene have been described to be associated with neurodegenerative diseases. Likewise, no experiments have been described that demonstrate a relationship between the dysregulation of the StAR gene and the pathology of neurodegenerative diseases, in particular Alzheimer's disease. The disclosures of the instant invention offer new ways, inter alia, for the diagnosis and treatment of said diseases.

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The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. A gene product comprises either RNA or protein and is the result of expression of a gene. The

amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). "Regulatory elements" shall comprise inducible and non-inducible promoters, enhancers, operators, and other elements that drive and regulate gene expression. The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or, acetylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene. The terms "agent", "reagent", or "compound" refer to any substance, chemical, composition or extract that have a positive or negative biological effect on a cell, tissue, body fluid, or within the context of any biological system, or any assay system examined. They can be agonists, antagonists, partial agonists or inverse agonists of a target. Such agents, reagents, or compounds may be nucleic acids, natural or synthetic peptides or protein complexes, or fusion proteins. They may also be antibodies, organic or anorganic molecules or compositions, small molecules, drugs and any combinations of any of said agents above. They may be used for testing, for diagnostic or for therapeutic purposes. The terms "oligonucleotide primer" or

"primer" refer to short nucleic acid sequences which can anneal to a given target polynucleotide by hybridization of the complementary base pairs and can be extended by a polymerase. They may be chosen to be specific to a particular sequence or they may be randomly selected, e.g. they will prime all possible sequences in a mix. The length of primers used herein may preferably vary from 10 nucleotides to 80 nucleotides. "Probes" are short nucleic acid sequences of the nucleic acid sequences described and disclosed herein or sequences complementary therewith. They may comprise full length sequences, or fragments, derivatives, isoforms, or variants of a given sequence. The identification of hybridization complexes between a "probe" and an assayed sample allows the detection of the presence of other similar sequences within that sample. The term "variant" as used herein refers to any polypeptide or protein, in reference to polypeptides and proteins disclosed in the present invention, in which one or more amino acids are added and/or substituted and/or deleted and/or inserted at the N-terminus, and/or the C-terminus, and/or within the native amino acid sequences of the native polypeptides or proteins of the present invention. Furthermore, the term "variant" shall include any shorter or longer version of a polypeptide or protein. "Variants" shall also comprise sequences that have at least about 80% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequences of the native polypeptides or proteins. "Variants" of a protein molecule include, for example, proteins with conservative amino acid substitutions in highly conservative regions. "Proteins and polypeptides" of the present invention include variants, fragments and chemical derivatives of a protein. They can include proteins and polypeptides which can be isolated from nature or be produced by recombinant and/or synthetic means. Native proteins or polypeptides refer to naturally-occurring truncated or secreted forms, naturally occurring variant forms (e.g. splice-variants) and naturally occurring allelic variants. In the present invention, the terms "risk", "susceptibility", and "predisposition" are tantamount and are used with respect to the probability of developing a neurodegenerative disease, preferably Alzheimer's disease. The term 'AD' shall mean Alzheimer's disease.

Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, ischemic stroke, age-related macular degeneration, and narcolepsy.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

The invention also relates to the construction and the use of primers and probes which are unique to the nucleic acid sequences of the StAR gene, or fragments or variants thereof, as disclosed in the present invention. The oligonucleotide primers and/or probes can be labeled specifically with fluorescent, bioluminescent, magnetic, or radioactive substances. The invention further relates to the detection and the production of said nucleic acid sequences, or fragments and variants thereof, using said specific oligonucleotide primers in appropriate combinations. PCR-analysis, a method well known to those skilled in the art, can be performed with said primer combinations to amplify said gene specific nucleic acid sequences from a sample containing nucleic acids. Such sample may be derived either from healthy or diseased subjects. Whether an amplification results in a specific

nucleic acid product or not, and whether a fragment of different length can be obtained or not, may be indicative for a neurodegenerative disease, in particular Alzheimer's disease. Thus, the invention provides nucleic acid sequences, oligonucleotide primers, and probes of at least 10 bases in length up to the entire coding and gene sequences, useful for the detection of gene mutations and single nucleotide polymorphisms in a given sample comprising nucleic acid sequences to be examined, which may be associated with neurodegenerative diseases, in particular Alzheimers disease. This feature has utility for developing rapid DNA-based diagnostic tests, preferably also in the format of a kit.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is ~~compared to a reference value representing a known disease or health status.~~ Thereby the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of

nucleic acid product or not, and whether a fragment of different length can be obtained or not, may be indicative for a neurodegenerative disease, in particular Alzheimer's disease. Thus, the invention provides nucleic acid sequences, oligonucleotide primers, and probes of at least 10 bases in length up to the entire coding and gene sequences, useful for the detection of gene mutations and single nucleotide polymorphisms in a given sample comprising nucleic acid sequences to be examined, which may be associated with neurodegenerative diseases, in particular Alzheimers disease. This feature has utility for developing rapid DNA-based diagnostic tests, preferably also in the format of a kit.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value representing a known disease or health status. Thereby the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a preferred embodiment, said subjects suffer from Alzheimer's disease.

The present invention discloses the differential expression and regulation of the StAR gene in specific brain regions of AD patients. Consequently, the StAR gene and its corresponding transcription and/or translation products may have a causative role in the regional selective neuronal degeneration typically observed in AD. Alternatively, StAR transcription and/or translation products may confer a neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular AD. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is particularly preferred that said sample to be analyzed and determined is ~~selected from the group consisting of a brain tissue or other tissues, organs~~ or body cells. The sample can also consist of cerebrospinal fluid or other body fluids such as saliva, urine, blood, serum plasma, or nasal mucosa.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an alteration in the level and/or activity of a ~~transcription product of the StAR gene and/or a translation product of the~~ StAR gene in a sample cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of becoming diseased with a neurodegenerative disease, particularly AD.

In preferred embodiments, the measurement of a level of a transcription product of the StAR gene is performed in a sample from a subject using a quantitative PCR-analysis with primer combinations to amplify said gene

specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A Northern blot with probes specific for said gene can also be applied. It might further be preferred to measure transcription products by means of chip-based microarray technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000).

Furthermore, a level and/or activity of a translation product of the StAR gene and/or fragment of said translation product, can be detected using an immunoassay, an activity assay and/or binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999). Enzymatic activity of a translation product of the StAR gene, may be measured by *in vitro*, cell-based, or *in vivo* assays. All of the above detection techniques may also be employed in the format of microarrays, protein-arrays, antibody microarrays, or protein-chip based technologies.

In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of the StAR gene, and/or of (ii) a translation product of the StAR gene, and/or of (iii) a fragment or derivative of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating neurodegenerative diseases, in particular AD, in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, said kit comprising:

- (a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the StAR gene (ii) reagents that selectively detect a translation product of the StAR gene; and
- (b) an instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of a subject to develop such a disease by
  - detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the StAR gene, in a sample from said subject; and

~~- diagnosing or prognosticating a neurodegenerative disease, in particular AD,~~  
or determining the propensity or predisposition of said subject to develop such a disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of a neurodegenerative disease, in particular AD, or an increased propensity or predisposition of developing such a disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease, in particular AD. Consequently, the kit, according to the invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in

particular AD, in a subject, as well as monitoring success or failure of therapeutic treatment for such a disease of said subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene, and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment or derivative of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence of a translation product of the StAR gene, or a fragment, or derivative, or a variant thereof.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane perturbation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against transcription products of the StAR gene. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy.

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be

performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection, liposomal mediated transfection.

In preferred embodiments, said agent is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector. In particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an AD-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment or derivative of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene, and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment or derivative of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment or derivative of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular AD.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for StAR, or a fragment thereof, or a derivative thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar to a neurodegenerative disease, in particular AD. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., 1994, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular AD.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene, and/or (iii) a translation product of the StAR gene, and/or (iv) a

fragment or derivative of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) the StAR gene, and/or (ii) a transcription product of the StAR gene, and/or (iii) a translation product of the StAR gene, and/or (iv) a fragment or derivative of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed said diseases and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses the StAR gene, or a fragment thereof, or a derivative thereof, under the control of a transcriptional regulatory element which is not the native StAR gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and a translation product of the StAR gene, or a fragment or derivative thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of a translation product of the StAR gene, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding fluorescently labelled ligand to said containers, and (iv) incubating said translation product of the StAR gene, or said fragment or derivative thereof, and said compound or plurality of compounds, and said fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said translation product of the StAR gene, or with said fragment or derivative thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said translation product of the StAR gene, or said fragment or derivative thereof. Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly.

Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a translation product of the StAR gene, or a fragment or derivative thereof. In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a translation product of the StAR gene by the aforementioned inhibitory binding assay and (ii) admixing the compound with

a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to a translation product of the StAR gene, or to a fragment or derivative thereof. Said screening assay comprises (i) adding a liquid suspension of a translation product of the StAR gene, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said translation product of the StAR gene, or said fragment or derivative thereof, and said fluorescently labelled compound or fluorescently labelled compounds, and (iv) measuring the amounts of fluorescence associated with said translation product of the StAR gene, or with said fragment or derivative thereof, and (v) determining the degree of binding by one or more of said compounds to said translation product of the StAR gene, or said fragment or derivative thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Said assay methods may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to a translation product of the StAR gene.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a StAR gene product by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a

medicament obtained by any of the methods according to the herein claimed screening assays.

The present invention features a protein molecule, said protein molecule being a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

The present invention also features a protein molecule, said protein molecule being a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the StAR gene, or a fragment, or derivative, or variant thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized, or single chain antibodies, as well as fragments thereof. Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods involving detecting translation products of the StAR gene.

In a preferred embodiment of the present invention, said antibodies can be used for detecting a pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, a pathological state relates to a neurodegenerative disease, in particular to AD. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described in US patent 6150173.

Other features and advantages of the invention will be apparent from the following description of figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in AD. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in AD (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in AD. Brain tissues from the frontal cortex (F) and the temporal cortex (T) of AD patients and healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figure 2 demonstrates the differential expression of the StAR gene in AD brain tissues by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and temporal cortex (T) of an AD patient (Fig 2A) and of a healthy, age-matched control individuals (Fig 2B) was performed by the LightCycler rapid thermal cycling technique. The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figure depicts the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of the StAR cDNA from both the frontal and temporal cortices of a normal control individual during the exponential phase of the reaction overlap (Fig 2B, arrow), whereas in AD (Fig 2A, arrows), there is a significant separation of the corresponding curves, indicating a differential expression of the StAR gene in the two analyzed brain regions.

Figure 3 charts the schematic alignment of StAR primer sequences to the nucleotide sequence of the human StAR gene (Sugawara et al., Proc. Natl. Acad. Sci. U.S.A. 1995, 92: 4778-4782; GenBank accession number U17280); polyA: polyadenylation site; primer positions P1, P2 are indicated by arrows; the open box represents the StAR open reading frame (positions 127-984).

Figure 4 shows the exact sequence alignments of the StAR primer pair to the nucleotide sequence of the human StAR gene (Sugawara et al., Proc. Natl. Acad. Sci. U.S.A. 1995, 92: 4778-4782; GenBank accession number U17280).

Table 1 lists the gene expression levels in the frontal cortex relative to the temporal cortex for the StAR gene in seven AD patients (1.72 to 7.30 fold) and five healthy, age-matched control individuals (0.59 to 2.25 fold). The values shown are reciprocal values according to the formula described herein (see below).

**EXAMPLE I:****(i) Brain tissue dissection from patients with AD:**

Brain tissues from AD patients and age-matched control subjects were collected within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Fig. 1) and stored at  $-80^{\circ}\text{C}$  until RNA extractions were performed.

**(ii) Isolation of total mRNA:**

Total RNA was extracted from post-mortem brain tissue by using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality were determined with the DNA LabChip system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were used to generate a melting curve with the LightCycler technology as described in the manufacturer's protocol (Roche).

**(iii) Quantitative RT-PCR analysis:**

The expression levels of the human StAR gene in temporal versus frontal cortex were analyzed using the LightCycler technology (Roche). This technique features rapid thermal cycling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by using a kinetic, rather than an endpoint readout. The ratio of StAR cDNA in frontal cortex versus temporal cortex was determined (relative quantification). First, a standard curve was generated to determine the efficiency of the PCR with specific primers for human StAR (5'-CCAATGTCAAGGAGATCAAGGTC-

3' and 5'-GCCAGCTCGTGAGTAATGAATGT-3'). PCR amplification (95 °C and 1 sec, 56 °C and 5 sec, and 72 °C and 5 sec) was performed in a volume of 20 µl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM MgCl<sub>2</sub>; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM MgCl<sub>2</sub>. Melting curve analysis revealed a single peak at approximately 80°C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 67 bp for StAR was observed in the electropherogram of the sample.

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTT-TGCC-3' except for MgCl<sub>2</sub> (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'-TTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM MgCl<sub>2</sub> was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83°C with no

visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number  $C_t$  for StAR and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from frontal cortex and temporal cortex were analyzed in parallel and normalized to cyclophilin B. The  $C_t$  values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10^{((C_t \text{ value} - \text{intercept}) / \text{slope})} \quad [\text{ng total brain cDNA}]$$

The values for frontal and temporal cortex cDNAs of StAR cDNA were normalized to cyclophilin B and the ratio was calculated according to formula:

$$\text{Ratio} = \frac{\text{StAR temporal [ng]} / \text{cyclophilin B temporal [ng]}}{\text{StAR frontal [ng]} / \text{cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from one gene to another gene remained constant in different runs, it was possible to normalize the values for StAR to the mean average value of the set of reference standard genes instead of normalizing to one single gene alone. The calculation was performed by dividing the ratio shown above by the

deviation of cyclophilin B from the mean value of all housekeeping genes. The results of one such quantitative RT-PCR analysis for the StAR gene are shown in Figure 2.

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## CLAIMS

1. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:

determining a level and/or an activity of

(i) a transcription product of the StAR gene, and/or

(ii) a translation product of the StAR gene, and/or

(iii) a fragment or derivative of said transcription or translation product

in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

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2. The method according to claim 1 wherein said neurodegenerative disease is Alzheimer's disease.

3. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the StAR gene and (ii) reagents that selectively detect a translation product of the StAR gene and

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(b) an instruction for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop such a disease by (i) detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the StAR gene, in a sample from said subject; and (ii) diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of said subject to develop

such a disease, wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status indicates a diagnosis or prognosis of a neurodegenerative disease, in particular Alzheimer's disease, or an increased propensity or predisposition of developing such a disease.

4. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

- (i) the StAR gene, and/or
- (ii) a transcription product of the StAR gene, and/or
- (iii) a translation product of the StAR gene, and/or
- (iv) a fragment or derivative of (i) to (iii).

5. A recombinant, non-human animal comprising a non-native gene sequence coding for StAR or a fragment thereof, or a derivative thereof, said animal being obtainable by:

- (i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and
- (ii) introducing said targeting construct into a stem cell of a non-human animal, and
- ~~(iii) introducing said non-human animal stem cell into a non-human embryo,~~  
and
- (iv) transplanting said embryo into a pseudopregnant non-human animal, and
- (v) allowing said embryo to develop to term, and
- (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and
- (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a

modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

6. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

- (i) the StAR gene, and/or
- (ii) a transcription product of the StAR gene, and/or
- (iii) a translation product of the StAR gene, and/or
- (iv) a fragment or derivative of (i) to (iii),

said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

(d) comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

7. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

- (i) the StAR gene, and/or
- (ii) a transcription product of the StAR gene, and/or
- (iii) a translation product of the StAR gene, and/or
- (v) a fragment or derivative of (i) to (iii),

said method comprising:

(a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative

disease or related diseases or disorders in respect of the substances recited in (i) to (iv);

- (b) measuring the activity and/or level of one or more substances recited in (i) to (iv);
- (c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched control animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;
- (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases or disorders.

8. The method according to claim 7 wherein said test animal and/or said control animal is a recombinant animal which expresses StAR, or a fragment thereof, or a derivative thereof, under the control of a transcriptional control element which is not the native StAR gene transcriptional control element.

9. An assay for testing a compound, preferably for screening a plurality of compounds for inhibition of binding between a ligand and a translation product of the StAR gene, or a fragment or derivative thereof, said assay comprising the steps of:

- (i) adding a liquid suspension of said translation product of the StAR gene, or a fragment or derivative thereof, to a plurality of containers;
- (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers;
- (iii) adding fluorescently labelled ligand to said containers;
- (iv) incubating said translation product of the StAR gene, or said fragment or derivative thereof, and said compound or compounds, and said fluorescently labelled ligand;

- (v) measuring amounts of fluorescence associated with said translation product of the StAR gene, or with said fragment or derivative thereof; and
- (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said translation product of the StAR gene, or said fragment or derivative thereof.

10. A protein molecule, said protein molecule being a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

11. A protein molecule, said protein molecule being a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

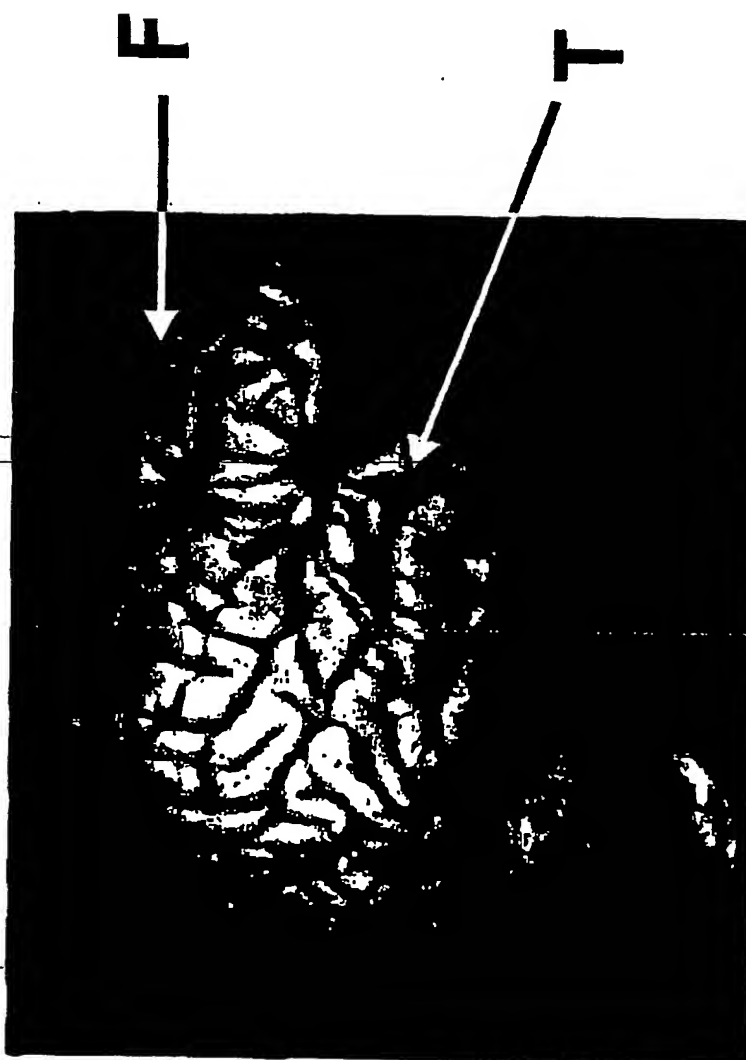
12. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the StAR gene, or a fragment, or derivative, or variant thereof, for detecting a pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell, and wherein said pathological state relates to a neurodegenerative disease, in particular Alzheimer's disease.

### SUMMARY

The present invention discloses the differential expression of the steroidogenic acute regulatory protein (StAR) gene in specific brain regions of Alzheimer's disease patients. Based on this finding, this invention provides a method for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or for determining whether a subject is at increased risk of developing such a disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using the StAR gene and gene products. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

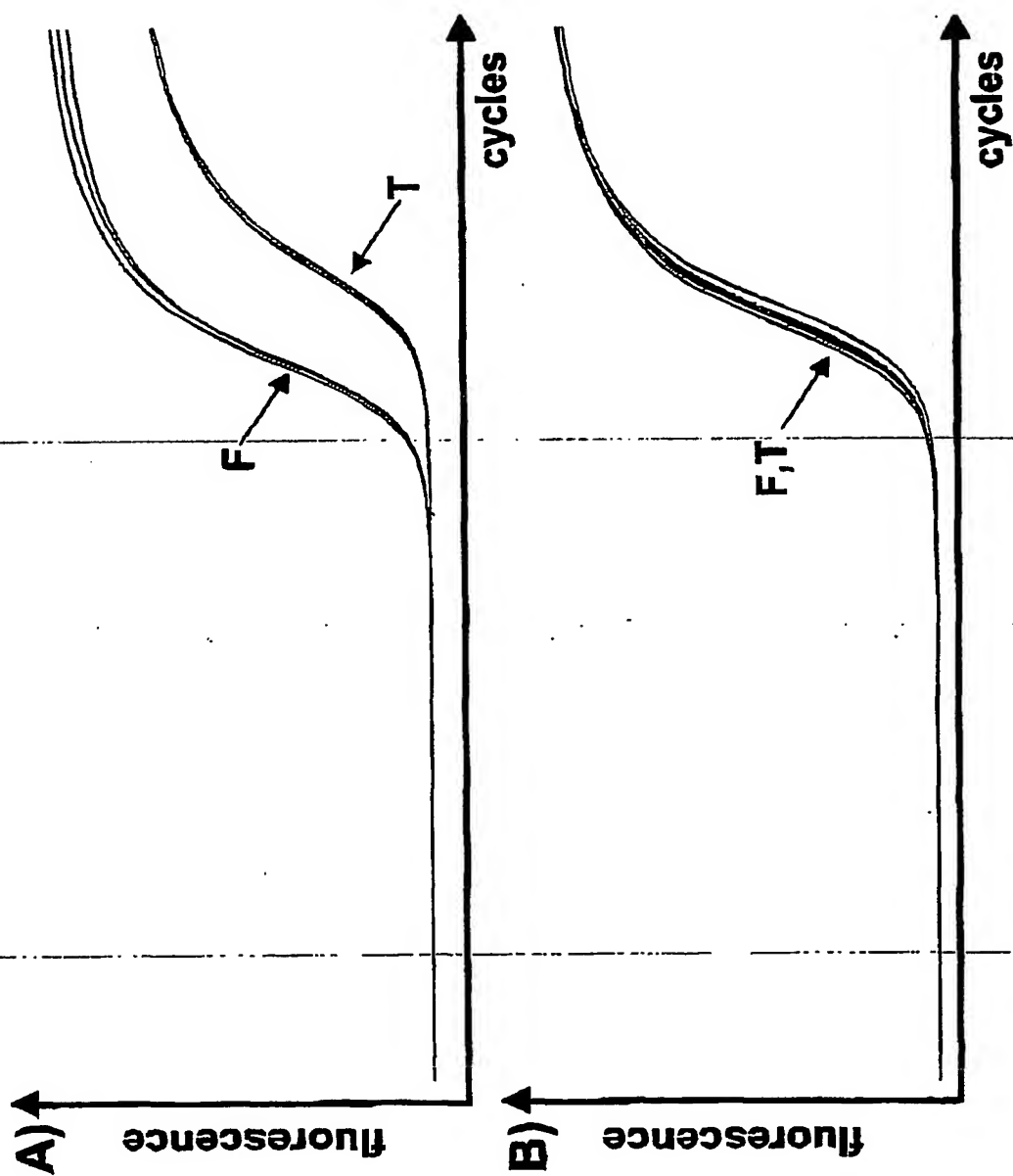
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**Figure 1: Identification of genes involved in  
Alzheimer's Disease pathology**



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**Figure 2: Differential expression of StAR as determined by RT-PCR analysis**



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**Figure 3: Schematic alignment of StAR RT-PCR primers with human StAR cDNA (GenBank accession number U17280)**



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**Figure 4: Alignment of StAR RT-PCR primers  
with StAR cDNA, GenBank accession number  
U17280**

```
1 CCAATGTCAAGGAGATCAAGGTC 23
  |||||
572 CCAATGTCAAGGAGATCAAGGTC 593
```

```
1 ACATTCATTACTCAGGAGCTGGC 23
  |||||
616 ACATTCATTACTCAGGAGCTGGC 638
```

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**Table 1: Differential expression of the StAR gene in the AD brain**

<b>sample</b>	<b><math>\Delta</math>fold (frontal / temporal cortex)</b>
<b>control 1</b>	<b>1.74</b>
<b>control 2</b>	<b>2.25</b>
<b>control 3</b>	<b>0.59</b>
<b>control 4</b>	<b>1.22</b>
<b>control 5</b>	<b>1.96</b>
<b>patient 1</b>	<b>6.16</b>
<b>patient 2</b>	<b>1.72</b>
<b>patient 3</b>	<b>2.00</b>
<b>patient 4</b>	<b>4.28</b>
<b>patient 5</b>	<b>2.36</b>
<b>patient 6</b>	<b>7.30</b>
<b>patient 7</b>	<b>6.23</b>

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